

15. The Poison-Organ of the Sting-Ray (*Trygon pastinaca*).
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(Text-figures 1-7.)

PART I. HISTORICAL SUMMARY.

The question of the presence of true poison-organs in fish is one which has exercised the minds of observers for centuries. From Aristotle down to the end of the nineteenth century the presence or absence of a poison-gland in the Sting-Ray has remained an unrevealed secret, although many observers have felt convinced that something besides the laceration by its serrated spines was necessary to cause the pain and inflammation that resulted from injuries produced by it. Even Bottard, to whom I am indebted for most of the early historical literature, denied the presence of a poison apparatus; and the 'Cambridge Natural History' merely states (p. 177) that "among Elasmobranchs the Eagle-Rays (*Aëtobatis*) and Sting-Rays (*Trygon*) have barbed or serrated spines on the tail, which inflict wounds far more severe than those caused by mere mechanical laceration; but, except the mucus secreted by the gland cells of the skin, which may possess venomous properties, no special poison-forming glands in connection with the spines are at present known."

Dr. Antonio Porta contributed a paper on venomous fish to the 'Anatomischer Anzeiger' of March 1, 1905. "It can be seen," he says (p. 235), "by what I have quoted above, that until now it was not known that a poison apparatus existed in the Trygonidae and Myliobatidae. The barb of the Trygon is almost similar to that of the Myliobatides, but it is longer and narrower. The said sting shows the lateral margins deceitfully serrated with the points turned from the back to the front. According to Moreau, in a fish of a medium size, the dart is very nearly one quarter of the length of the back, but there is nothing exact in its proportion. In nine specimens (*T. violacea* and *T. pastinaca*) of medium size that I examined, I found that the length of the dart varied from 8·7 to 12·6 cm. It is renewed every year of the life of the fish, and since sometimes the new one sprouts before the old one falls off, we find individual fish armed with two or, more rarely, three or four stings. If we isolate a sting and examine it, we observe on the ventral aspect two grooves on either side of a ridge which become shallow in width and depth towards the base. In these two grooves the poison-organs are situated, which penetrate to the deepest part of the groove and there continue laterally and above into two small

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tubes which converge at the base, providing a passage for the blood capillaries, which supply the connective tissue surrounding the gland."

"On making transverse sections of the sting and putting them under the microscope, we see that the ventral furrows are occupied by a glandular mass of a more or less triangular shape with the corners rounded off. It is composed of a great many cells of various sizes and shapes ($\cdot 2$ – $\cdot 4$ mm. $\times 510$), often joined to one another to form true glandular follicles, which measure $\cdot 7$ – $1\cdot 5$ mm. $\times 510$; the connecting and surrounding tissues are rich in blood-vessels and communicate with the sheath of the sting. Towards the apex the gland gets smaller, the cells become less numerous and smaller and are surrounded by much connective tissue, with which they gradually merge."

"This gland is similar to that which is observed in the genus *Scorpena* and in the greater number of other poisonous fish. It should be considered as a cutaneous gland. The dart is merely an arm of defence. It is united to the tail by strong ligaments and muscles, which only, however, permit a small lateral movement. The emission of the poison takes place in a very simple manner. The sting introducing itself into the wound, the sheath is drawn back towards the base and presses on the gland which thus emits a poisonous liquid, which flowing towards the narrow apical groove thus inoculates the wound."

PART II. OBSERVATIONS ON SERIAL SECTIONS.

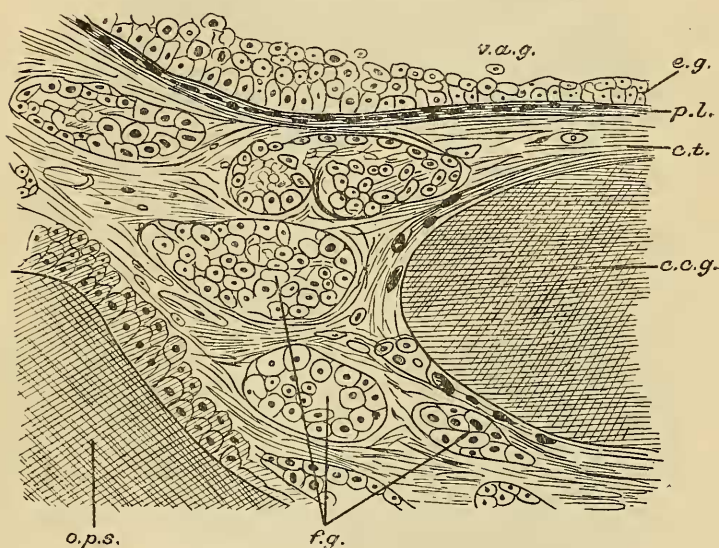
I am in agreement with Dr. Porta as to the position and general triangular outline of the gland. I have not personally examined the poison-organ of *Scorpena*, but the elongated compressed cells pictured by Bottard in the grooves of the spine of *Scorpena* are of the same type as the gland-cells of both *Trachinus draco* and *vipera*, and according to that authority the poison-gland of *Scorpena* is a less developed type of the gland found in the Weevers. Having myself made many sections of the glands of both the Great and Lesser Weevers, I can state with assurance that the glandular structure found in *Trygon* is of a totally different type: in fact, there are many points in its structure of a unique character, and the arrangement of the cells requires careful examination. The gland consists, for the most part, of a fine mesh, within the interstices of which are groups of small cells with a vacuolated protoplasm. These cells are grouped together in regular follicles.

These follicles in other parts are entirely filled with secretion, so that you have a cystic appearance, a distended cavity, lined by a layer of flattened cells. The external margin of this glandular mass shows a well-marked layer of pigment-cells, and external to this are several layers of rounded epithelial cells, which, however, are frequently detached in the sections, as shown in text-fig. 2.

If we could understand the origin and relations to other parts

of the tissues which occupy the lateral grooves, which for convenience and brevity I propose in future to speak of as the glandular triangle (Porta speaks of it having a triangular shape in section with the angles rounded off), we must study sections at the root of the spine or dart, before it separates from the whip-like tail. With the naked eye one can see a special dark pigmented patch on the tail where it opposes the spine, on the surface of which appears some soft whitish epidermis. Moreover, if we view the spine at this point in profile, we notice that

Text-figure 1.

*Trygon pastinaca.*

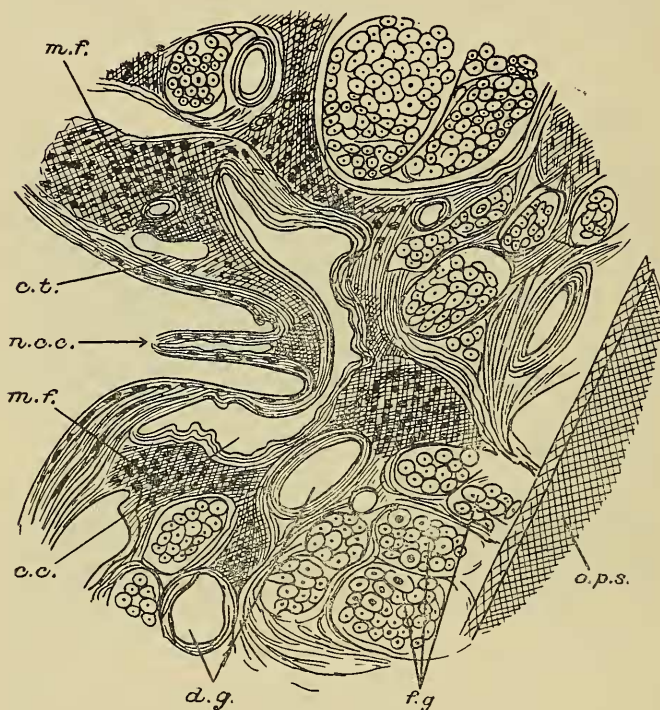
Part of gland in groove.

c.c.g. Central canal of groove. *c.t.* connective tissue. *e.g.* epithelium of groove.
f.g. follicles of gland. *o.p.s.* osseous part of spine. *p.l.* pigment-layer.
v.a.g. ventral aspect of groove.

the dorsum of the spine becomes free of the epidermis which has been covering it sooner than the ventral aspect, so that the dorsum of the spine in this respect is somewhat similar to the nail on a man's finger. The epidermis ends rather abruptly on the dorsum, while, on the other hand, the dentate margin and the lateral grooves between it and the ventral ridge separate gradually from the tail, the last part to become free being the ventral ridge. On either side there is a gradual invagination of ectoderm between the tail and the glandular triangle, this

invagination becoming deeper as one traces it posteriorly. Microscopically, one can observe that this invagination is carried out by a specialised portion of epithelium. Where the spine begins to separate, the flattened epidermal layer and mucous cells covering the tail are replaced by a layer of columnar cells

Text-figure 2.

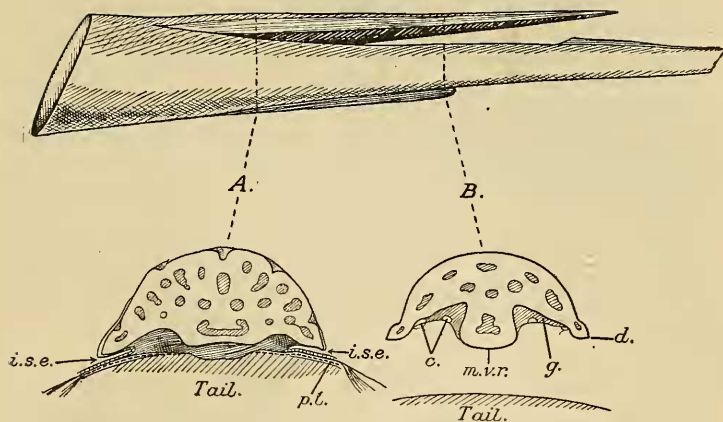
*Trygon pastinaca.*

Portion of gland of groove, showing follicles full of secreting cells, and central canal empty.

c.c. Central canal or duct (empty). *c.t.* connective tissue. *d.g.* ducts of gland. *f.g.* follicles of gland. *m.f.* muscle-fibres. *n.c.c.* nipple of central canal. *o.p.s.* osseous portion of spine.

with superimposed layers of rounded cells, resting on a basement-membrane in which is a layer of large deeply pigmented cells. This peculiar layer of epithelial tissue gradually grows inwards, *i. e.*, towards the middle line on either side, and inserts itself between the tissues occupying the grooves and the tail.

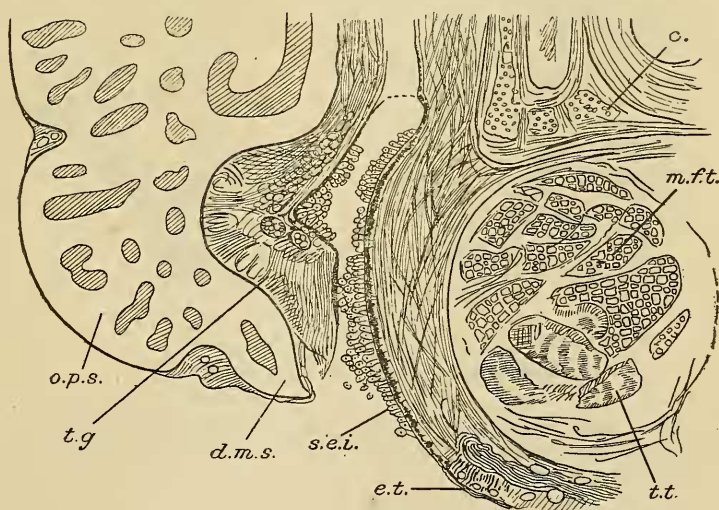
Text-figure 3.

*Trygon pastinaca.*

Lateral view of spine, with diagrammatic sections at A, B.

c. Canals. d. tooth. g. glandular tissue. i.s.e. invagination of specialised epithelium. m.v.r. median ventral ridge. p.l. pigment-layer.

Text-figure 4.

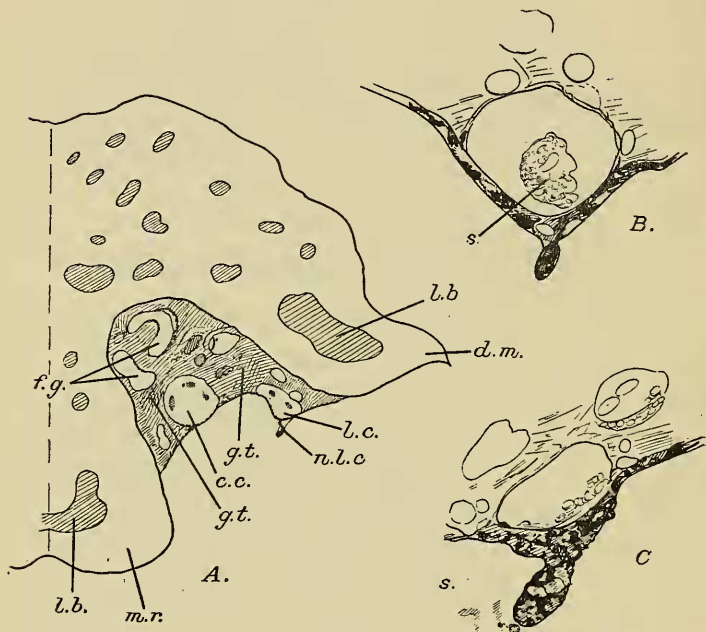
*Trygon pastinaca.*

Half-section of spine separating from tail, showing invagination of specialised epithelium and pigment-layer.

c. Cartilage. d.m.s. dentate margin of spine. e.t. ordinary epithelium of tail, m.f.t. muscle-fibres of tail. o.p.s. osseous portion of spine. s.e.i. special epithelium of invagination. t.g. tissue of groove. t.t. tendon of tail.

The tissue of the triangle at this stage consists of the fibrous tissue which precedes the formation of bone; the pigmented layer and the round cells in this way extend over the tissue of the groove, and the actual separation of the spine from the tail is accomplished by a division of the round-celled epithelium, so that when the separation is complete there is a pigment-layer covering the glandular triangle covered with several layers of epithelium, and a similar pigment-layer with epithelial covering facing it on the dorsal surface of the tail.

Text-figure 5.

*Trygon pastinaca.*

- A. Transverse section of spine near base.
 B. Lateral canal and nipple. Canal full.
 C. " " " Canal emptying.

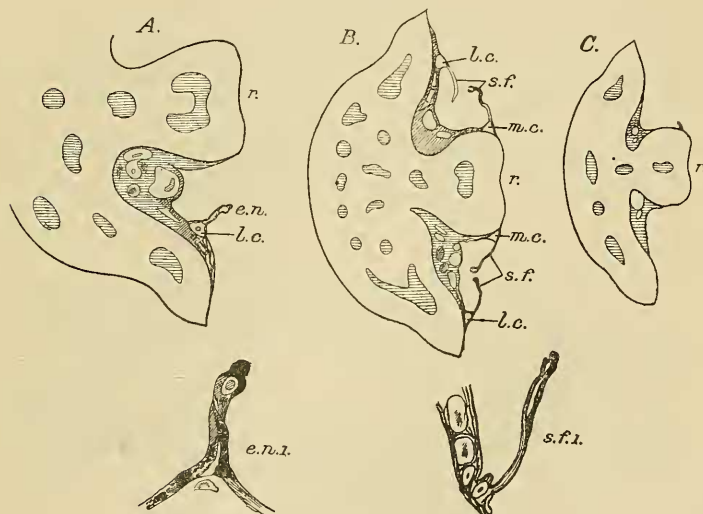
c.c. Central canal. *d.m.* dentate margin. *f.g.* follicles of gland. *g.t.* glandular tissue.
l.b. lacunae of bone. *l.c.* lateral canal. *m.r.* median ridge. *n.l.c.* nipple of lateral canal. *s.* secretion.

The pigment-layer may also be observed to dip into the triangular area, and carries with it these rounded cells.

If we now examine more carefully the secreting tissue of the glandular triangle, we notice throughout the length of the groove, but more markedly near its base, two definite rounded

cavities, occupied by a mass of homogeneous yellow material staining yellow by Van Giesen's method. These cavities tend throughout the whole series of sections to arrange themselves into two ducts or canals, one lying near the centre of the gland or towards the ridge, and the other lying towards the lateral margin in the direction of the teeth. The follicles seem to empty themselves either into one canal or the other. As each duct or canal becomes filled with secretion it bulges the pigment-layer so as to make it protrude externally. On the peripheral margin of the lateral canal this bulge is surmounted by a curious nipple-shaped projection which appears throughout the whole series of sections, at times distended, at times empty and flattened. Some of the sections show the nipple discharging a fluid from its

Text-figure 6.

*Trygon pastinaca.*

A-C. Series of sections progressively diminishing towards tip.

e.n. Elongated nipple. *e.n.1.* the same enlarged (note bulbous tip containing secretion). *l.c.* lateral canal. *m.c.* canal of median ridge (*r.*). *s.f.* secreting filaments. *s.f.1.* the same enlarged.

tip, and one feels convinced that these sections actually show the secretion from the lateral canal being discharged externally. Towards the tip of the spine, where the groove is smaller, the nipple is prolonged into a filamentous tube.

By counting the sections in series and observing the presence or absence of a nipple-like projection from the lateral canal, one is able to estimate approximately the size of these projections.

A nipple will extend for .3-.4 mm., and there is then a gap of about .2 mm. until the next nipple appears on the lateral canal. In this way the dentate margin is moistened with secretion, and as the teeth lacerate the tissue of the victim the poison becomes inoculated in the wound. Towards the tip of the spine there is little glandular tissue: the central canal divides into two or more channels, and the secretion is discharged by means of a canal, of which there are two on either side, a lateral canal near the base of the teeth, and a canal resting on the margin of the median ridge where it looks towards the lateral groove.

Text-figure 7.

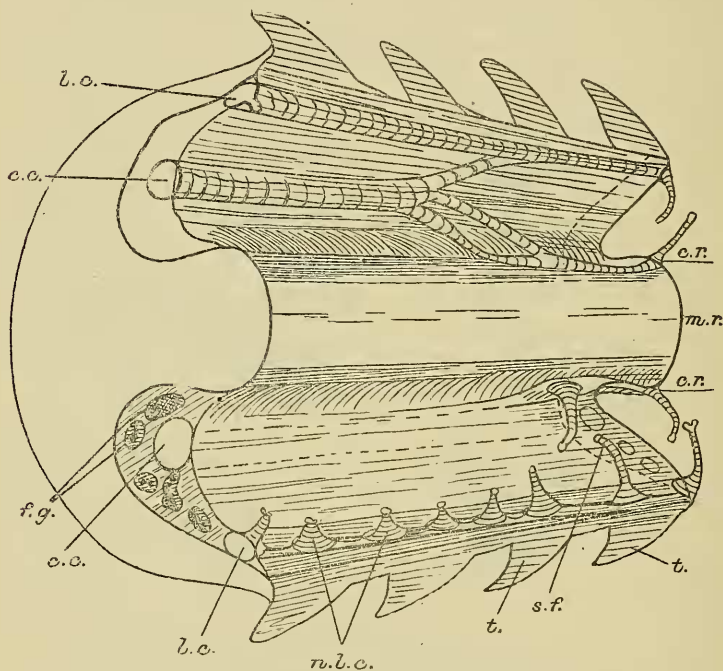
*Trygon pastinaca.*

Diagram of portion of spine to show scheme of canals and relations of nipples and filaments.

c.c. Central canal. c.r. canal of ridge. f.g. follicles of gland. l.c. lateral canal. m.r. median ridge. n.l.c. nipples of lateral canal. s.f. secreting filaments. t. tooth pointing forwards.

It is here that these lateral canals give off the hollow filamentous tubes, which project towards each other and are of such length that the two make a bridge across the lateral groove. They

terminate by an orifice which is curiously pigmented, and some sections show the secretion issuing from the tip.

The number of these secreting filaments varies, and apparently they are provided in order to carry the poison to the tip of the dart, where the glandular tissue no longer is present. The width of a filament is from 2 to 3 mm.

It should be mentioned that in one spine of the three of which I have sections, there is a well-marked nipple projecting from the central canal near the base (see text-fig. 2), and the canal can be seen surrounded by a layer of muscular fibres.

A diagram of this arrangement of glandular tissue, central and lateral canals, nipples and secreting filaments, is given in text-fig. 7; it is purely diagrammatic, and does not give with any pretence of accuracy the actual relative size of the nipples compared with the teeth of the dentate margin.

In concluding this account of the microscopic anatomy of the spine, I must mention with gratitude the help I have received from Dr. Stuart McDonald, of the University of Durham, whose laboratory assistant, Mr. Percy Landreth, took the microphotographs from which several of the illustrations have been prepared, and who gave me much technical help.

I must also mention the kindness of Mr. C. Tate Regan, of the British Museum (Natural History), in looking at my sections, in giving me references to the literature on the subject, and making suggestions as to the form this paper should take.

I must also thank those members of the Board of Agriculture and Fisheries, including Mr. Borley, for the help they have given me in obtaining specimens.

In conclusion, I would add that my investigations began in 1911, and were undertaken in ignorance of the work of Dr. Porta, who, so far as I can ascertain, was the first to describe the gland of the groove in the spine of *Trygon*.

Summary of the evidence that the gland is really a poison-organ:—

- i. That the nature of the wounds produced are not such as would happen after a simple laceration;
- ii. that the symptoms of acute pain and inflammation are similar to the symptoms produced by the stings of the other venomous fish, particularly the weever;
- iii. that the staining reactions of the secretion are similar to the staining reactions of the poison of *Trachinus draco*;
- iv. the observations of Dr. Lo Bianco quoted by Dr. Porta.

The observations of Dr. Lo Bianco are very interesting. He himself saw a young man become extremely pale and fall down almost senseless for a few minutes, from having received only a very small puncture while he was in the act of passing a *Trygon* weighing 3 kg. from one person to another. Besides which he also relates the following most interesting fact. In the month of

September there were in the great tank of the Aquarium of the Zoological Station of Naples four *Trygon violacea* and three *Thalassochelys caretta*. One of the Trygons died, and on examining it he found that the sting was broken and entirely gone. After a few days one of the *Thalassochelys* would not eat any more, unlike the others who ate with great appetite, and remained in a corner of the tank: it lived thus for four days and died on the fifth. On examining it he found the sting of the *Trygon* buried quite 6 cm. under its right fin, piercing only the skin and muscles; in the part where the sting was buried the tissue was of a violet colour. The wound was about 3 to 4 cm. in length and breadth, and contained a putrid liquid with a most offensive smell.

The results of this investigation show:—

- i. The origin of the gland from a special layer of epithelium starting at the root of the spine.
- ii. That the secreting tissue consists of regular follicles with ducts and central and lateral canals.
- iii. That the secretion is discharged by means of nipples or filaments projecting from the canals.
- iv. That there is a layer of muscular tissue surrounding the central canal.

Works consulted.

1. BOTTARD'S "Les Poissons venimeux," 1889.
2. KOBERT'S "Giftfische und Fischgifte," 1902.
3. PORTA'S paper in the "Anatomischer Anzeiger," xxvi. 1905.
4. CALMETTE'S "Les Venins," 1907.
5. Cambridge Natural History, Fishes, 1904.

There are full bibliographies in Bottard's work and in Porta's paper.